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(54) Title: AEROSOL FORMULATIONS OF PEPTIDES AND PROTEINS

(57) Abstract

A pharmaceutical aerosol formulation comprising (a) a HFA propellant; (b) a pharmaceutically active polypeptide dispersible in the propellant; and (c) a surfactant which is a C_8-C_{16} fatty acid or salt thereof, a bile salt, a phospholipid, or an alkyl saccharide, which surfactant enhances the systemic absorption of the polypeptide in the lower respiratory tract.

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AEROSOL FORMULATIONS OF PEPTIDES AND PROTEINS

This invention relates to drug formulations containing medically useful peptides and proteins, for inhalation from an aerosol inhaler.

Background of the Invention

A range of drugs are administered in aerosol formulations through the mouth or nose. One widely used method for dispensing such an aerosol formulation involves making a suspension formulation of the drug as a finely divided powder in a liquefied gas known as a propellant. Pressurised metered dose inhalers, or pMDI's, are normally used to dispense such formulations to a patient. Surface active agents, or surfactants, are commonly included in order to aid dispersion of the drug in the propellant and to prevent aggregation of the micronised drug particles.

Until recently, chlorofluorocarbon-containing propellants (CFC's) were accepted for use in all pharmaceutical aerosol formulations. Typical surfactant dispersing agents used in the CFC formulations were for example sorbitantrioleate, oleic acid, lecithines, and ethanol. Since CFC's have been implicated in the destruction of the ozone layer, a new generation of propellants has emerged to take their place.

Hydrofluoroalkane (HFA) propellants such as 1,1,1,2-tetrafluoroethane (P134a), 1,1,1,2,3,3,3-heptafluoropropane (P227) and 1,1-difluoroethane (P152a) are considered to be the most promising new propellants. Not only are they environmentally acceptable, but they also have low toxicity and vapour pressures suitable for use in aerosols. However the surfactants normally used in CFC-aerosol formulations are not particularly suitable for use with the new generation of propellants and therefore in recent years a number of alternative surfactants have been suggested for use specifically with the HFA propellants, among them polyethoxylated surfactants and fluorinated surfactants.

Peptide-based drugs have not traditionally been among those drugs which are administered from aerosol formulations, although various aerosol formulations have been suggested. For example US Patent number 5,284,656 discloses a formulation of granulocyte colony stimulating factor (G-SCF) comprising a finely divided powder containing G-SCF suspended in a propellant, with the aid of a surfactant such as sorbitan trioleate, soya lecithin or oleic acid. US Patent number 5,364,838 discloses an insulin formulation wherein a dry powder of insulin is suspended within a low boiling point propellant with an excipient such as oleic acid.

Summary of the Invention

We have now surprisingly found that various substances which enhance the absorption of polypeptides in the respiratory tract are also particularly suitable as surfactants for use with HFA propellants.

The invention thus provides a pharmaceutical aerosol formulation comprising (a) a HFA propellant; (b) a pharmaceutically active polypeptide dispersible in the propellant; and (c) a surfactant which is a C_8-C_{16} fatty acid or salt thereof, a bile salt, a phospholipid, or an alkyl saccharide, which surfactant enhances the systemic absorption of the polypeptide in the lower respiratory tract.

The surfactants employed in the present invention are surprisingly suitable for use with HFA propellants; their capabilities for enhancement of the absorption of polypeptide give them a dual function which makes them especially beneficial for use in the present polypeptide aerosol formulations.

Of the fatty acids and salts thereof, C_8-C_{16} fatty acids salts are preferred. Examples of preferred fatty acid salts are sodium, potassium and lysine salts of caprylate (C_8), caprate (C_{10}), laurate (C_{12}) and myristate (C_{14}). As the nature of the counterion is not of special significance, any of the salts of the fatty acids are potentially useful. A particularly preferred fatty acid salt is sodium caprate.

Suitable bile salts may be for example salts of cholic acid, chenodeoxycholic acid, glycocholic acid, taurocholic acid, glycochenodeoxycholic acid, taurochenodeoxycholic acid, deoxycholic acid, glycodeoxycholic acid, taurodeoxycholic acid, lithocholic acid, and ursodeoxycholic acid.

Of the bile salts, trihydroxy bile salts are preferred. More preferred are the salts of cholic, glycocholic and taurocholic acids, especially the sodium and potassium salts thereof. The most preferred bile salt is sodium taurocholate.

Suitable phospholipids may be for example single-chain phospholipids, for example lysophosphatidylcholine, lysophosphatidylglycerol, lysophosphatidylethanolamine, lysophosphatidylinositol and lysophosphatidylserine or double-chain phospholipids, for example diacylphosphatidylcholines, diacylphosphatidylglycerols, diacylphosphatidylethanolamines, diacylphosphatidylinositols and diacylphosphatidylserines.

Of the phospholipids, diacylphosphatidylglycerols and diacylphosphatidylcholines are preferred, for example dioctanoylphosphatidylglycerol and dioctanoylphosphatidylcholine.

Suitable alkyl saccharides may be for example alkyl glucosides or alkyl maltosides, such as decyl glucoside and dodecyl maltoside.

The most preferred surfactants are bile salts.

The propellant may comprise one or more of 1,1,1,2-tetrafluoroethane (P134a), 1,1,1,2,3,3,3-heptafluoropropane (P227) and 1,1-difluoroethane (P152a), for example, optionally in admixture with one or more other propellants. Preferably the propellant comprises P134a or P227, or a mixture of P134a and P227, for example a density-matched mixture of p134a and P227.

The polypeptide may be any medically or diagnostically useful peptide or protein of small to medium size, i.e. up to about 40 kD molecular weight (MW), for which systemic delivery is desired. The mechanisms of improved polypeptide absorption according to the present invention are generally applicable and should apply to all such polypeptides, although the degree to which their absorption is improved may vary according to the MW and the physico-chemical properties of the polypeptide, and the particular surfactant used. It is expected that polypeptides having a molecular weight of up to 30 kD will be most useful in the present invention, such as polypeptides having a molecular weight of up to 25 kD or up to 20 kD, and especially up to 15 kD or up to 10kD.

The polypeptide is preferably a peptide hormone such as insulin, glucagon, C-peptide of insulin, vasopressin, desmopressin, corticotropin (ACTH), corticotropin releasing hormone (CRH), gonadotropin releasing hormone (GnRH), gonadotropin releasing hormone agonists and antagonists, gonadotrophin (luteinizing hormone, or LHRH), calcitonin, parathyroid hormone (PTH), bioactive fragments of PTH such as PTH(34) and PTH(38), growth hormone (GH) (for example human growth hormone (hGH)), growth hormone releasing hormone (GHRH), somatostatin, oxytocin, atrial natriuretic factor (ANF), thyrotropin releasing hormone (TRH), deoxyribonuclease (DNase), prolactin, and follicle stimulating hormone (FSH), and analogues of any of the above.

Other possible polypeptides include growth factors, interleukins, polypeptide vaccines, enzymes, endorphins, glycoproteins, lipoproteins, and polypeptides involved in the blood coagulation cascade, that exert their pharmacological effect systemically. It is expected that most if not all polypeptides of small to medium size can be effectively delivered by the methods of the invention.

The preferred polypeptide is insulin.

In addition to drug, propellant and surfactant, a small amount of ethanol (normally up to 5% but possibly up to 20%, by weight) may be included in the formulations of the present

invention. Ethanol is commonly included in aerosol compositions as it can improve the function of the metering valve and in some cases also improve the stability of the dispersion.

The composition may of course contain other additives as needed, including other pharmaceutically active agents, adjuvents, carriers, flavouring agents, buffers, antioxidants, chemical stabilisers and the like. As examples of suitable additives may be mentioned for example lactose, glucose, fructo::e, galactose, trehalose, sucrose, maltose, raffinose, maltitol, melezitose, stachyose, lactitol, palatinite, starch, xylitol, mannitol, myoinositol, and the like, and hydrates thereof, and amino acids, for example alanine, glycine and betaine, and peptides and proteins, for example albumen.

The preferred carrier is melezitose.

The formulation of the present invention is particularly advantageous because of the dual function of the particular surfactants employed. The surfactants as provided in the present invention are not only surprisingly capable of producing fine dispersions in the new generation of propellants, but, very importantly, also enhance polypeptide absorption. The present formulations are stable and bioavailability of the polypeptides is high, with good reproducibility.

The surfactants used in the present invention may enhance the absorption of the polypeptide by for example

(1) Enhancement of the paracellular permeability of a polypeptide by inducing structural changes in the tight junctions between the epithelial cells.

(2) Enhancement of the transcellular permeability of a polypeptide by interacting with or extracting protein or lipid constituents of the membrane.

(3) Interaction between enhancer and polypeptide which increases the solubility of the polypeptide in aqueous solution. This may occur by preventing formation of polypeptide

aggregates (dimers, trimers, hexamers), or by solubilizing polypeptide molecules in enhancer micelles.

(4) Decreasing the viscosity of, or dissolving, the mucus barrier lining the alveoli and passages of the lung, thereby exposing the epithelial surface for direct absorption of the polypeptide.

(5) Reducing the activity of protease inhibitors in the lungs, thereby increasing the stability of the polypeptide, increasing absorption.

The surfactants may function by only a single mechanism set forth above, or by two or more. A surfactant acting by several mechanisms is more likely to promote efficient absorption of a polypeptide than one which employs only one or two.

By "enhances absorption" is meant that the amount of polypeptide absorbed into the systemic circulation in the presence of surfactant is higher than in its absence.

Preferably the surfactant is present in the present invention in a surfactant : polypeptide ratio in the range of approximately 1:10 to 1:0.2, preferably 1:4 to 1:1, more preferably 1:4 to 1:2.5. The preferred concentration of polypeptide in the formulations of the present invention is 0.1 mg/ml to 25 mg/ml.

As much as possible of the polypeptide preferably consists of particles having a diameter of less than 10 microns, for example 0.01-10 microns or 0.1-6 microns, for example 0.1-5 microns. Preferably at least 50% of the polypeptide consists of particles within the desired size range. For example at least 60%, preferably at least 70%, more preferably at least 80% and most preferably at least 90% of the polypeptide consists of particles within the desired size range.

Therefore, the polypeptide for use in the present invention may have to be processsed prior to inclusion in the formulations, in order to produce particles in the desired size range. For example the polypeptide may be micronised, for example in a suitable mill, such as a jet

mill. Alternatively, particles in the desired particle range may be obtained by for example spray drying or controlled crystallisation methods, for example crystallisation using supercritical fluids.

Preferably, the surfactant for use in the present invention also consists of particles within the desired size range. Suitably, the polypeptide and surfactant may be mixed in an aqueous buffer and dried to give a solid powder which is then optionally micronised. The micronised powder may then be added to a fraction of the propellant (and optional ethanol) at low temperature. After mixing in of the drug the remaining surfactant and propellant and optionally ethanol may be added and the suspension filled into appropriate containers.

The polypeptide aerosol formulation of the present invention is useful for the local or systemic treatment of diseases and may be administered for example via the upper and lower respiratory tract, including by the nasal route. As such the present invention also provides said polypeptide aerosol formulation for use in therapy; the use of the polypeptide aerosol formulation in the manufacture of a medicament for the treatment of diseases via the respiratory tract; and a method for the treatment of a patient in need of therapy, comprising administering to said patient a therapeutically effective amount of the polypeptide aerosol formulation of the present invention.

The diseases which may be treated with the polypeptide aerosol formulation of the present invention are any of those which may be treated with the particular polypeptide in each case; for example formulations containing insulin according to the present invention may be used for example in the treatment of diabetes; formulations containing corticotropin may be used for example in the treatment of inflammatory diseases; formulations containing GnRH may be useful for example in the treatment of male infertility. The indications for all of the mentioned polypeptides are well known. The polypeptide aerosol formulations of the present invention may also be used in prophylatic treatment.

The following Examples are intended to illustrate, but not limit, the invention:

Formulations of insulin in P134a and/or P227 with different surfactants were prepared in order to assess the quality of the suspensions formed. In the following examples the quality of the suspension is rated as "acceptable" or "good". An acceptable suspension is characterised by one or more of slow settling or separation, ready re-dispersion, little flocculation, and absence of crystallisation or morphology changes, such that the dispersion is sufficiently stable to give a uniform dosing. A good dispersion is even more stable.

Example 1

Method

Insulin (25-35 parts, as below) was added to a beaker with water and dissolved. Surfactant (65-75 parts, as below) was added and dissolved and the pH was adjusted to 7.4 The solution was concentrated by evaporation of the water. The obtained solid cake was crushed, sieved and micronised in a jet mill. 40 or 50 mg of the powder was added to a plastic coated glass bottle. The bottle was chilled to approximately -40°C with a mixture of carbon dioxide ice and isopropanol, and 10 ml chilled P134a (at approximately -40°C) was added. The bottle was sealed with a metering valve and then shaken vigorously for about 30 seconds. Examples 1g to 1n were additionally treated in an ultrasonic bath for about 10 minutes.

Results

Example No.	Surfactant	Ratio surfactant:insulin	Suspension
1a	sodium caprate	25:75	good
1b	potassium caprate	27:73	good
1c	lysine caprate	35:65	good
1d	sodium myristate	30:70	good
1e	sodium laurate	25:75	good
1f	sodium caprylate	25:75	good
1g	sodium taurocholate	25:75	good

Example No.	Surfactant	Ratio surfactant:insulin	Suspension
1h	dioctanoylphosphatitidyl glycerol	25:75	good
1j	dodecylmaltoside	25:75	good
1k	lysopalmitoylphosphatidyl glycerol	25:75	acceptable
1m	lysopalmitoylphosphatidyl choline	25:75	acceptable
1n	dioctanoylphosphatidyl choline	25:75	good

Example 2

Sodium caprate (25 parts) and insulin (75 parts) were micronised separately and then dry mixed. 40 mg of this mixture was added to a plastic coated glass bottle. The bottle was chilled to approximately -40°C with a mixture of carbon dioxide ice and isopropanol, and 10 ml chilled (approximately -40°C) P134a was added. The bottle was sealed with a metering valve and then shaken vigorously for about 30 seconds. A good suspension formed.

Example 3

Method

Insulin (25-35 parts, as below) was added to a beaker with water and dissolved. Surfactant (65-75 parts, as below) was added and dissolved and the pH was adjusted to 7.4. The solution was concentrated by evaporation of the water. The obtained solid cake was crushed, sieved and micronised in a jet mill. 40 or 50 mg of the powder was added to a plastic coated glass bottle. The bottle was chilled to approximately -40°C with a mixture of carbon dioxide ice and isopropanol, and 10 ml chilled (approximately -40°C) P227 was added. The bottle was sealed with a metering valve and then shaken vigorously for about

30 seconds. Examples 3g to 3n were additionally treated in an ultrasonic bath for about 10 minutes.

Results

Example No.	Surfactant	Ratio surfactant:insulin	Suspension
3a	sodium caprate	25:75	good
3b	potassium caprate	27:73	good
3c	lysine caprate	35:65	good
3d	sodium myristate	30:70	good
3e	sodium laurate	25:75	good
3f	sodium caprylate	25:75	good
3g	sodium taurocholate	25:75	good
3h	dioctanoylphosphatitidyl glycerol	25:75	good
3j	dodecylmaltoside	25:75	good
3k	lysopalmitoylphosphatidyl glycerol	25:75	acceptable
3m	lysopalmitoylphosphatidyl choline	25:75	acceptable
3n	dioctanoylphosphatidyl choline	25:75	good

Example 4

Micronised formulations containing DNase, surfactant (sodium taurocholate or dioctanoylphosphatidylglcerol), and melezitose (ratio DNase : surfactant : melezitose 1 : 0.33 : 98.67, total weight 50 mg), were added to a plastic coated glass bottle, chilled to approximately -40°C. Chilled propellant 134a or propellant 227 (approximately -40°C,

approximately 10 ml) was added and the bottles sealed with a metering valve and treated in an ultrasonic bath for approximately 10 minutes.

Identical formulations were prepared to which 5%(w/w) of ethanol was added prior to the treatment in the ultrasonic bath.

The quality of the suspensions formed were assessed immediately and after storage at room temperature for 20 hours. In all cases good suspensions were observed.

Example 5

Sodium caprate and insulin were micronised separately and then dry mixed. The proportion of sodium caprate to insulin was 25:75 . 80mg of this mixture was added to an aerosol vial. The aerosol vial was chilled to approximately -40°C with a mixture of carbon dioxide ice and isopropanol, and 10 ml chilled (approximately -40°C) P134a was added. The aerosol vial was sealed with a 50 µl metering valve and then treated for a couple of minutes in an ultra sonic bath.

The particle size of insulin in the aerosol delivered from the container was measured with an "Andersen" impactor at 28.3 lit/min. The fraction of fine particles (less than 6 µm) was 41% of the delivered dose. The particle measurement was repeated after storage for two months at room temperature and no deterioration was observed. The fine particle fraction was 46%.

Example 6

50 aerosol units containing insulin and sodium taurocholate (ratio 75:25, 8mg/ml) were prepared as follows : The substances were weighed into a beaker. The beaker was chilled to approximately -40°C with a mixture of carbon dioxide and isopropanol. Propellant (p134a, approximately -40°C) was added, the mixture was mixed for a few minutes with an ultraturrax, then poured into a manufacturing vessel, and further propellant (p134a, approximately -40°C) was added. (Total volume 500 ml) The formulation was stirred

with an ultraturrax and filed into metered dose inhalers, 10 ml in each. The inhalers were sealed with metering valves.

The aerosols were stored in varying conditions:

5°C, dry conditions	for 2, 8, and 13 weeks
30°C, 30% relative humidity	for 11 weeks

The quality of the suspensions assessed. In all cases good suspensions were observed.

In addition the stability of the insulin was assessed by measuring, using standard chromatographical techniques, the concentration of the degradation products desamido insulin and other insulin-related impurities. In all cases the level of impurities was within acceptable limits (less than 5% desamido insulin and less than 3% other insulin-related impurities).

Example 7

A pressurised metered dose inhaler filled with the preparation of Example 5 was actuated, and the delivered aerosol collected in a spacer. An airflow (8 lit/min) was led through the spacer into the delivery system, to which each of five dogs were exposed for five minutes. The target inhaled dose was 1U.insulin/kg. The bioavailability was determined by comparison of the plasma curve after inhalation and the plasma curve after intravenous injection from earlier studies. The bioavailability was 66% of the drug reaching the lungs.

Example 8

Pressurised metered dose inhalers were filled with the formulation of Example 6 or with a corresponding formulation without enhancer. Each inhaler was actuated, and the delivered aerosol collected in a spacer. An airflow (8 lit/min) was led through the spacer into the delivery system, to which each of five dogs were exposed for two minutes. The target inhaled dose was 1U.insulin/kg. Blood samples were collected before dosing and at

various time intervals up to 245 minutes after the start of dosing. The plasma insulin concentration was measured by radioimmunoassay.

From the formulations without enhancer, the insulin was in general absorbed relatively slowly, with peak plasma insulin levels occuring between 50 and 100 minutes after administration is some of the animals. In other animals peak plasma insulin levels occured between 10 and 20 minutes after administration.

From the formulations according to the invention, a peak plasma insulin level was reached in all animals within 10 minutes of adminstration, followed by another peak at around 25 minutes, in all animals.

The generally faster absorption of insulin from the formulations according to the invention is closer to the natural insulin plasma curve following meals, in healthy people. Moreover the lack of variation between animals indicates that a desired level of insulin absorption is easier and more reliably achieved using the formulations of the present invention.

Claims

1. A pharmaceutical aerosol formulation comprising (a) a HFA propellant; (b) a pharmaceutically active polypeptide dispersible in the propellant; and (c) a surfactant which is a C_8-C_{16} fatty acid or salt thereof, a bile salt, a phospholipid, or an alkyl saccharide, which surfactant enhances the systemic absorption of the polypeptide in the lower respiratory tract.

2. A pharmaceutical aerosol formulation as claimed in claim 1, wherein the surfactant is a C_8-C_{16} fatty acid salt.

3. A pharmaceutical aerosol formulation as claimed in claim 2, wherein the fatty acid salt is selected from the sodium, potassium and lysine salts of caprylate (C_8), caprate (C_{10}), laurate (C_{12}) and myristate (C_{14}).

4. A pharmaceutical aerosol formulation as claimed in claim 1, wherein the surfactant is a trihydroxy bile salt.

5. A pharmaceutical aerosol formulation as claimed in claim 4, wherein the bile salt is selected from the salts of cholic, glycocholic and taurocholic acids.

6. A pharmaceutical aerosol formulation as claimed in claim 5, wherein the bile salt is selected from the sodium and potassium salts of cholic, glycocholic and taurocholic acids.

7. A pharmaceutical aerosol formulation as claimed in claim 6, wherein the bile salt is sodium taurocholate.

8. A pharmaceutical aerosol formulation as claimed in claim 1, wherein the surfactant is a single-chain phospholipid.

9. A pharmaceutical aerosol formulation as claimed in claim 8, wherein the surfactant is selected from lysophosphatidylcholines, lysophosphatidylglycerols, lysophosphatidylethanolamines, lysophosphatidylinositols and lysophosphatidylserines.

10. A pharmaceutical aerosol formulation as claimed in claim 1, wherein the surfactant is a double-chain phospholipid.

11. A pharmaceutical aerosol formulation as claimed in claim 11, wherein the surfactant is selected from diacylphosphatidylcholines, diacylphosphatidylglycerols, diacylphosphatidylethanolamines, diacylphosphatidylinositols and diacylphosphatidylserines.

12. A pharmaceutical aerosol formulation as claimed in claim 11, wherein the surfactant is selected from dioctanoylphosphatidylglycerol and dioctanoylphosphatidylcholine.

13. A pharmaceutical aerosol formulation as claimed in claim 1, wherein the sufactant is selected from alkyl glucosides and alkyl maltosides.

14. A pharmaceutical aerosol formulation as claimed in claim 13, wherein the surfactant is selected from decyl glucoside and dodecyl maltoside.

15. A pharmaceutical aerosol formulation as claimed in any of claims 1-14, wherein the propellant comprises 1,1,1,2-tetrafluoroethane (P134a), 1,1,1,2,3,3,3-heptafluoropropane (P227) or 1,1-difluoroethane (P152a).

16. A pharmaceutical aerosol formulation as claimed in claim 15, wherein the propellant comprises 1,1,1,2-tetrafluoroethane (P134a) and 1,1,1,2,3,3,3-heptafluoropropane (P227).

17. A pharmaceutical aerosol formulation as claimed in claim 15 or 16, wherein the propellant comprises a density-matched mixture of 1,1,1,2-tetrafluoroethane (P134a) and 1,1,1,2,3,3,3-heptafluoropropane (P227).

18. A pharmaceutical aerosol formulation as claimed in any preceding claim, wherein the polypeptide is of molecular weight up to 40 kD.

19. A pharmaceutical aerosol formulation as claimed in claim 18, wherein the polypeptide is of molecular weight up to 30 kD.

20. A pharmaceutical aerosol formulation as claimed in claim 19, wherein the polypeptide is of molecular weight up to 25 kD.

21. A pharmaceutical aerosol formulation as claimed in any preceding claim, wherein the polypeptide is of molecular weight up to 20 kD.

22. A pharmaceutical aerosol formulation as claimed in any preceding claim, wherein the polypeptide is of molecular weight up to 15 kD.

23. A pharmaceutical aerosol formulation as claimed in any preceding claim, wherein the polypeptide is of molecular weight up to 10kD.

24. A pharmaceutical aerosol formulation as claimed in any preceding claim, wherein the polypeptide is a peptide hormone.

25. A pharmaceutical aerosol formulation as claimed in any of claims 1-17, wherein the polypeptide is selected from insulin, glucagon, C-peptide of insulin, vasopressin, desmopressin, corticotropin (ACTH), corticotropin releasing hormone (CRH), gonadotropin releasing hormone (GnRH), gonadotropin releasing hormone agonists and antagonists, gonadotrophin (luteinizing hormone, or LHRH), calcitonin, parathyroid

hormone (PTH), bioactive fragments of PTH such as PTH(34) and PTH(38), growth hormone (GH) (for example human growth hormone (hGH)), growth hormone releasing hormone (GHRH), somatostatin, oxytocin, atrial natriuretic factor (ANF), thyrotropin releasing hormone (TRH), deoxyribonuclease (DNase), prolactin, and follicle stimulating hormone (FSH), and analogues thereof.

26. A pharmaceutical aerosol formulation as claimed in claim 25, wherein the polypeptide is insulin.

27. A pharmaceutical aerosol formulation as claimed in any preceding claim, including ethanol in an amount of up to 20% by weight of propellant and surfactant.

28. A pharmaceutical aerosol formulation as claimed in any preceding claim, including ethanol in an amount of up to 5% by weight of propellant and surfactant.

29. A pharmaceutical aerosol formulation as claimed in any preceding claim, including additives selected from adjuvents, carriers, flavouring agents, buffers, antioxidants and chemical stabilisers.

30. A pharmaceutical aerosol formulation as claimed in claim 29, wherein the additive is selected from lactose, glucose, fructose, galactose, trehalose, sucrose, maltose, raffinose, maltitol, melezitose, stachyose, lactitol, palatinite, starch, xylitol, mannitol, myoinositol, hydrates thereof, alanine, glycine and betaine, and albumen.

31. A pharmaceutical aerosol formulation as claimed in claim 30, wherein the carrier is melezitose.

32. A pharmaceutical aerosol formulation as claimed in any preceding claim, wherein the surfactant is present in a surfactant : polypeptide ratio in the range of 1:10 to 1:0.2.

33. A pharmaceutical aerosol formulation as claimed in claim 32, wherein the surfactant is present in a surfactant : polypeptide ratio in the range of 1:4 to 1:1

34. A pharmaceutical aerosol formulation as claimed in any preceding claim, wherein the polypeptide comprises particles having a diameter of 0.01-10 microns.

35. A pharmaceutical aerosol formulation as claimed in claim 34, wherein the polypeptide comprises particles having a diameter of 0.1-6 microns.

36. A pharmaceutical aerosol formulation as claimed in claim 34, wherein the polypeptide comprises particles having a diameter of 0.1-5 microns.

37. A pharmaceutical aerosol formulation as claimed in any of claims 34-36, wherein at least 50% of the polypeptide consists of particles within the said size range.

38. A pharmaceutical aerosol formulation as claimed in any of claims 34-36, wherein at least 60% of the polypeptide consists of particles within the said size range.

39. A pharmaceutical aerosol formulation as claimed in any of claims 34-36, wherein at least 70% of the polypeptide consists of particles within the said size range.

40. A pharmaceutical aerosol formulation as claimed in any of claims 34-36, wherein at least 80% of the polypeptide consists of particles within the said size range.

41. A pharmaceutical aerosol formulation as claimed in any of claims 34-36, wherein at least 90% of the polypeptide consists of particles within the said size range.

42. A pharmaceutical aerosol formulation as claimed in any preceding claim, wherein the concentration of polypeptide is 0.1 mg/ml to 25 mg/ml of the formulation.

43. A method for the manufacture of a polypeptide aerosol formulation as claimed in any of claims 1-42, comprising the steps of: mixing the polypeptide and the surfactant in an aqueous buffer; drying to give a solid powder; optionally micronising the solid powder; adding the optionally micronised powder, propellant and optional ethanol to a vessel at low temperature; mixing; and adding further propellant and optional ethanol.

44. A polypeptide aerosol formulation as claimed in any of claims 1-42, for use in therapy.

45. The use of a polypeptide aerosol formulation as claimed in any of claims 1-42 in the manufacture of a medicament for the treatment of diseases via the respiratory tract.

46. A method for the treatment of a patient in need of therapy, comprising administering to said patient a therapeutically effective amount of the polypeptide aerosol formulation as claimed in any of claims 1-42.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/01540

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 9/12, A61K 47/12, A61K 47/24, A61K 47/26, A61K 47/28, A61K 38/28
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, WPIL, CLAIMS, EMBASE, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 9116038 A1 (TORAY INDUSTRIES, INC.), 31 October 1991 (31.10.91) --	1-45
Y	EP 0518600 A1 (SCHERING CORPORATION), 16 December 1992 (16.12.92) -- --------	1-45

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 1996	02 -04- 1996

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 94/01540

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 46
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest.
[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

05/02/96

International application No.
PCT/SE 95/01540

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO-A1-	9116038	31/10/91	EP-A-	0477386	01/04/92
			JP-A-	5000963	08/01/93
EP-A1-	0518600	16/12/92	AU-A-	2017592	12/01/93
			CA-A-	2111002	23/12/92
			CN-A-	1067578	06/01/93
			CZ-A-	9302714	13/07/94
			EP-A-	0588897	30/03/94
			EP-A-	0656206	07/06/95
			EP-A-	0656207	07/06/95
			FI-D-	935464	00/00/00
			HU-A-	67449	28/04/95
			JP-T-	6511235	15/12/94
			NO-A,D-	934500	09/12/93
			OA-A-	9868	15/08/94
			SK-A-	140493	05/10/94
			US-A-	5474759	12/12/95
			WO-A-	9222288	23/12/92